The object of the study is microorganisms isolated from the Azerbaijani territory of the Caspian Sea, capable of decomposing oil and oil products at low temperatures of 4–6 °C. The purpose of this work was to study bacteria and fungi capable of actively assimilating oil, gasoline, kerosene, diesel fuel at a temperature of 4–6 °C in order to solve the problem of environmental pollution in this region.

The studied most effective biodestructors are combined into associations that can be used to create an active biological product based on native microorganisms with its further use for water bioremediation, as well as treatment of tanks and other containers used for long-term storage of petroleum products at a temperature of 4–6 °C.

The process of degradation of samples of used oils at the molecular level of the selected compounds was studied by reverse-phase high-performance liquid chromatography. As a result of chromatographic and spectroscopic studies, it was found that almost all microorganisms decompose the tested oil products. Only weak peaks remained on the chromatogram, which is a clear indicator of the deep biodegradation carried out by these microorganisms. Thanks to the chromatographic and spectral analysis of oil and products of its decomposition, an oxidation sequence has been established, indicating the decomposition of the last aromatic fraction. As a result, a general picture was obtained on the amount of degradation products containing carboxyl, keto-, hydroxyl groups, which is not enough to determine the processes of transformation of initial products into final ones. All this testifies to the multidirectionality of biodegradation processes

Keywords: biodegradation of microorganisms, oil and oil products, Caspian Sea, low temperature, liquid chromatography

UDC 579.22
DOI: 10.15587/1729-4061.2023.287467

DEGRADATION OF OIL AND OIL PRODUCTS BY MICROORGANISMS ISOLATED FROM THE AZERBAIJANI COAST OF THE CASPIAN SEA AT LOW TEMPERATURES

Aynur Babashli
Candidate of Biological Sciences, Associate Professor*
Nazilya Akhundova
Corresponding author
Candidate of Biological Sciences, Associate Professor*
E-mail: nazilya.akhundova@mail.ru
Natavan Gadimova
Candidate of Technical Sciences, Associate Professor*
*Department of Engineering and Applied Sciences
Azerbaijan State University of Economics (UNEC)
İstiglaliyyat str., 6, Baku, Azerbaijan, AZ1001

Received date 05.07.2023
Accepted date 12.09.2023
Published date 30.10.2023

How to Cite: *Babashli, A., Akhundova, N., Gadimova, N. (2023). Degradation of oil and oil products by microorganisms isolated from the Azerbaijani coast of the Caspian sea at low temperatures. Eastern-European Journal of Enterprise Technologies, 5 (10 (125)), 17–24. doi: https://doi.org/10.15587/1729-4061.2023.287467*

1. Introduction

Today, as a result of anthropogenic activity, there is a large-scale pollution of the environment with toxic substances. The action of specialized modified factors on hydroecosystems affects the degradation of the habitat of hydrobionts, which leads to the disruption of interdependent relationships in hydrobiocenoses [1]. It is known that untreated or poorly treated urban wastewater causes pollution of water bodies [2].

The use of a complex of mechanical, chemical and physico-chemical cleaning methods is not always acceptable due to the threat of secondary pollution. Relevant is the search for environmentally friendly methods to eliminate the consequences of pollution with petroleum hydrocarbons, aimed at enhancing the metabolic activity of microorganisms capable of oxidizing organic toxicants to safe products, using them as a source of carbon and energy.

Oil and oil products are recognized as the main environmental pollutants. Petroleum hydrocarbons, falling into one of the natural spheres, participate in the general migration of substances and spread in each of them [3]. Oil and oil products are one of the main contributors to environmental pollution worldwide. Accidental spills of oil and oil products cause significant damage to ecosystems and lead to negative economic and social consequences [4].

Since at the present level, with the development of the oil industry, it is not possible to completely eliminate its negative impact on the environment, it becomes necessary to develop methods and technologies for cleaning up the environment polluted with petroleum hydrocarbons. The mechanical and chemical methods used to remove hydrocarbons from contaminated areas are of limited effectiveness and can be expensive. The efficiency of using acoustic cavitation for disinfection and purification of natural waters of various reservoirs is shown. After the treatment of polluted water with ultrasound, there was a decrease in the magnitude of chemical and biological contamination of water [5].

Bioremediation is mainly based on biodegradation, which can refer to the complete mineralization of organic pollutants into carbon dioxide, water, inorganic compounds and cellular protein, or to the conversion of complex organic pollutants to other simpler organic compounds by biological agents such as microorganisms. Many microorganisms are able to decompose hydrocarbon pollutants [6, 7]. Self-purification of natural waters from pollution is a complex process that occurs under the influence of physical, chemical and biological factors, in which the leading role belongs to microorganisms of various taxonomic groups, mainly bacteria and micromycetes [8].

The Caspian Sea is considered the largest lake on earth with salt water. Since ancient times, the southern part of the

Caspian Sea – Azerbaijan has been subjected to oil pollution. The amount of pollutants in the Caspian Sea undergoes significant seasonal changes under the influence of hydrometeorological, chemical and biological factors, the largest amount of oil occurs in the winter period, the smallest in the summer. As we mentioned above, the main role in cleaning up the contaminated area belongs to the biological factor – microorganisms involved in the transformation of petroleum hydrocarbons. Due to its economic performance and environmental safety, the use of biological methods is preferable to physical and chemical methods in the purification of water bodies from oil pollution. Although some studies have been carried out on the destruction of oil and oil products at a temperature of 24–28 °C using oil-absorbing microorganisms isolated off the coast of Azerbaijan, scientific studies on the destruction of oil and oil products by these microorganisms at low temperatures have not been carried out at all. In this regard, it is especially important to study the destruction of oil and oil products at temperatures of 4–6 °C.

2. Literature review and problem statement

The paper [9] shows that the combined use of oil-degrading bacteria and natural polymers with the properties of surfactants and humic acids in bioorganic compositions is effective for cleaning up the degradation of oil and oil products in the environment. But the issues related to the choice of the correct ratios of reagents and (humic acids and bacteria) are still not resolved. Correct selection can be an option to overcome the corresponding difficulties. This is the approach used in [10].

The paper [11] shows that the main role in self-cleaning is played by microorganisms that oxidize hydrocarbons to end products – carbon dioxide and water. But as before, issues related to oil degraders have not been resolved. The reasons for this may be low temperature and low bioavailability of hydrophobic oil hydrocarbons, which make it difficult to clean up oil-contaminated areas of reservoirs. An option for overcoming the corresponding difficulties may be a certain selection of an association of microorganisms adapted to lower temperature conditions.

It was shown in [12] that temperature is a significant factor influencing the ability of microorganisms to destroy oil and petroleum hydrocarbons. For the development of oil-decomposing bacteria and their intensification of the process of destruction of hydrocarbons, mesophilic conditions are optimal, that is, 20–28 °C. But issues related to temperature are still not resolved, at a temperature of 6–15 °C, the intensity of oil transformation decreases by 2.5–4 times. The reasons for this may be a decrease in water temperature for every 5 °C, which is accompanied by a disproportionate decrease in the biological activity of microorganisms. This limits the processes of water purification at low temperatures, for example, in the autumn-winter period. An option to overcome the corresponding difficulties can be the use of psychrophilic bacterial species for this experiment, which are resistant to lower temperatures. All this allows us to assert the expediency of a repeated seasonal study.

The paper [13] shows the study of the biodegradation of hydrocarbons in thin films of oil in sea water at low temperatures of 0 and 5 °C. The results of DGGE sequencing and analysis indicated temperature differences in oil-associated bacterial communities and showed that the predominant types in seawater attach to oil-coated adsorbents. Both characterization of community members from enriched ODM cultures and DGGE bands from biodegradation experiments showed a predominance of proteobacterial sequences. However, sequence analysis did not reveal the identity of bacterial populations in ODM cultures and DGGE bands. The reasons for this may be the conditions of cultivation and experiments on biodegradation, which differed significantly. An option to overcome the corresponding difficulties may be to improve the cultivation conditions and repeat the experiments. All this suggests that it is advisable to conduct repeated studies on the biodegradation of hydrocarbons in thin films of oil in sea water at low temperatures of 0 and 5 °C.

In [14], 4 strains belonging to the genera Rhodococcus and Pseudomonas, which destroy 40 % of oil, were isolated, and the degradation of oil, fuel oil, benzene, and ethanolbenzene at 4–6 °C was studied using them. It was shown that the maximum destruction of fuel oil and ethanolbenzene was observed in a strain belonging to the genus *Pseudomonas* and amounted to 17.2 and 5.2 **%**. But in strains belonging to the genus *Rhodococcus*, it was 16.6 **%**. The reason for the stability of petroleum products in the environment is their limited solubility in aqueous media. Therefore, such compounds are inaccessible to microorganisms and are more difficult to biodegrade. An option to overcome the corresponding difficulties can be the use of microorganisms of various physiological groups capable of synthesizing bioemulsifiers, thereby increasing the bioavailability of oil and oil products. All this suggests the expediency of conducting a study on the ability of microorganisms to emulsify, which is one of the defining functions in the process of oil degradation.

The paper [15] compared the degradation of diesel fuel and oil at temperatures of 4–6 °C and 24 °C by 96 strains of oil-degrading microorganisms and found that the degree of oil degradation in 6 strains is higher at temperatures of 4–6 °C and 24 °C. However, there are no results of the study of decomposition products. All this suggests that it is advisable to conduct repeated studies.

The paper [16] shows the ability of individual microbial cultures to decompose aliphatic and aromatic series of oil hydrocarbons of the Caspian Sea in the area of the Apsheron Peninsula. Liquid fractions of paraffins (C4–C10) and some individual hydrocarbons (butane, pentane, hexane), as well as some individual aromatic hydrocarbons (toluene, styrene, isopropylbenzene, methylstyrene), were used as the only carbon source in the research experiments on biodegradability. The work was not the purpose of studying microorganisms at low temperatures.

All this allows us to assert that it is expedient to study the biodegradation of oil and oil products, as well as biodegradation products at a temperature of 4–6 °C by microorganisms isolated from the shores of the Caspian Sea, therefore our research is concentrated in this area.

3. The aim and objectives of the study

The aim of this work was to screen active psychotropic hydrocarbon-oxidizing microorganisms isolated from the waters of the Caspian Sea and to identify the possibilities of using isolated cultures that can effectively utilize oil and its fractions (diesel fuel, kerosene, gasoline) to purify waters contaminated with oil products at a temperature of 4–6 °C, to create biological products for the bioremediation of oil-contaminated waters and determine its effectiveness in laboratory conditions.

To achieve this aim, the following objectives were accomplished:

– to isolate and identify active psychotropic microorganisms that destroy petroleum hydrocarbons;

– to make microbial associations based on a set of physiological and metabolic properties with selection under conditions of periodic cultivation with oil as a substrate;

– to study the destructive processes of used oil samples from a molecular perspective.

4. Materials and methods of research

The object of this study was bacterial strains and micromycetes isolated from the waters and soils of the Caspian Sea.

The hypothesis of the study suggests the possibility of using our methodology to study the biodegradation of oil and oil products by microorganisms isolated from the Caspian Sea.

To isolate strains of bacteria – oil degraders, the samples were incubated on a shaker at temperatures of 4–6 °C in a liquid elective nutrient medium: NH_4NO_3 – 1.0 g, K_2HPO_4 – 1.0 g, KH_2PO_4 – 1 g, KH_2PO_4 – 1g, $MgSO_4$ – 0.2 g, $CaCl_2$ – 0.02 g, 2 drops, saturated solution of $FeCl_2$, NaCl – 13 g with the addition of oil by weight of 2 % per liter of water [8]. The activity of the isolated strains was determined by various indicators: turbidity of the medium, film formation, gas evolution and precipitation. Then the resulting enrichment cultures were transferred to an agar medium to obtain pure cultures of oil-oxidizing microorganisms. The generic composition of the isolated bacteria was established on the basis of their morphological and physiological-biochemical characteristics according to the Bergey's determinant [17]. Agar Capek medium was used to isolate micromycetes.

The work also revealed the ability of active oil-oxidizing bacteria to absorb oil and oil products. For this, oil, kerosene, gasoline and diesel fuel were used as the only source of carbon. Micromycetes for seed materials were grown on agar jamb for 1–2 weeks at temperatures of 4–6 °C. The cells were washed off with 10 ml of distilled water, then 5 ml of the resulting suspension were added to the flasks. Micromycetes were grown in flasks with a capacity of 0.25 l, containing 0.10 l of liquid selective medium, at temperatures of 4–6 °C, antibiotics were added to the medium to inhibit the growth of bacteria. Oil, kerosene, gasoline and diesel fuel were used as the sole source of carbon. 1–2 day cultures grown on MPA were used as inoculum of bacteria. The cells were washed off with 10 ml of distilled water, then 5 ml of the resulting suspension was added to the flasks. In all experiments, cultivation was carried out at a temperature of 4–6 °C. Bacteria were grown in the above mentioned medium in flasks with a capacity of 0.25 l with 0.1 l of nutrient medium containing oil, kerosene, gasoline, fuel oil and diesel fuel as the only source. The culture fluid, freed from cells by filtration, was extracted with an equal volume of hexane. The destruction process of the selected compounds was studied by reversed-phase high-performance liquid chromatography.

The structural transformations of the studied samples occurring during biodegradation were studied by chromatographic analysis (by the method of reversed-phase liquid chromatography). A liquid chromatograph of the company "Kovo" (Czech Republic), with UV-spectrophotometric detectors with an operating wavelength of λ=254 nm was used; two chromatographic columns with a size of 3.3×150 mm, filled with a reversed stationary phase "Separon SGX-C18", with a particle size of 7 μm, medium temperature 20–25 °C; mobile phase hexane: isopropyl alcohol (2:98 vol.%), stationary phase rate 0.3 ml/min were used. The components were identified by comparing the retention parameters of the standard mixture and the biotransformation products. Standard solutions with a concentration of 1–1.5 mg/mL were prepared in the elution system of methanol: water (75:25 vol.%).

The structural composition of the biodegradation of oil and oil products was determined by the following methods: IR spectroscopy (UR-20) (thin layer) in the spectral range of 4,000–700 cm^{-1}. For all experiments, control experiments were carried out (without the introduction of biodestructors of bacteria and fungi).

5. Results of destructors of oil and petroleum products at low positive temperatures

5. 1. Isolation and identification of active strains of oil-degrading microorganisms

As a result of research, 21 strains of bacteria and 26 strains of fungi were identified from water samples taken from the coast of the Caspian Sea in the winter-spring period. Morphological, cultural and biochemical features of the selected strains are identified to genus. Identified active strains of bacteria: *Bacillus, Micrococcus, Pseudomonas, Arthrobacter, Acinetobacter, Aeromonas, Vibrio, Sarcina, Alcaligenes.* Fungi are classified as *Aspergillus, Penicillium, Mucor, Fusarium, Trichoderma.* Then we studied the ability of isolated active strains of microorganisms to absorb oil and oil products at 4–6 °C temperatures. Of the isolated strains of micromycetes, 96 % grew on oil, 82 % on kerosene, 60 % on diesel fuel and 54 % on gasoline (Fig. 1).

While 70 % of the isolated bacterial strains used oil as the only source of energy and carbon, 43 % used kerosene. 58 % of microorganisms developed in diesel fuel, 34 % in gasoline (Fig. 2).

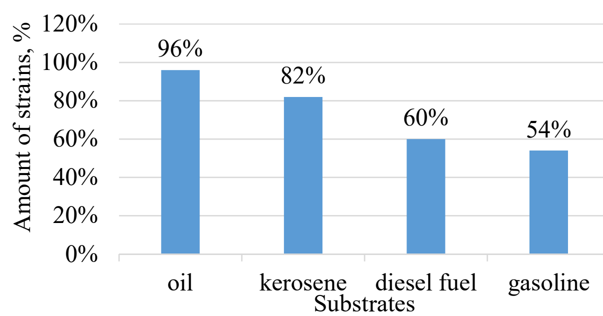

Fig. 1. Growth of micromycetes on various substrates

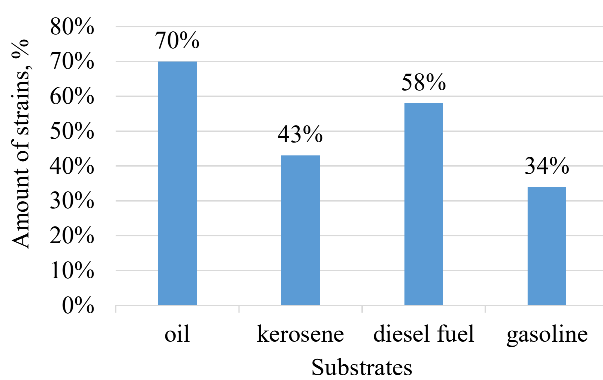

Fig. 2. Growth of bacteria on various substrates

The experiments performed showed that the compounds used were suitable as the only source of carbon for the growth of each of the isolated groups of microorganisms.

5. 2. Selection of active strains for association

Associations of active strains of microorganisms were created and their biodegradation of oil and oil products was studied at a temperature of 4–6 °C.

When creating associations, we took into account the activity of microorganisms, more active strains of micromycetes (Aspergillus sp.1, Aspergillus sp.2, Penicillium sp.4, Mucor sp.3, Fusarium sp.2) and bacteria (Bacillus sp.1, Bacillus sp.2, Pseudomonas sp.3, Pseudomonas sp.4, Arthrobacter sp.2, Acinetobacter sp.1) were selected and included in the association (Table 1).

Table 1

The ability of some active strains of microorganisms to absorb oil and petroleum products

Microorganisms	Substrate			
	Oil	Kerosene	Gasoline	Diesel fuel
Bacillus sp.1	+++	+++	+++	++
Bacillus sp.2	+++	+++	+++	++
Bacillus sp.3	++	++	+++	-
Micrococcus sp.1	++	++	++	+
Micrococcus sp.2	++	++	++	–
Pseudomonas sp.1	++	+++	+++	+
Pseudomonas sp.2	++	+	++	++
Pseudomonas sp.3	+++	+++	+++	++
Pseudomonas sp.4	+++	++	+++	++
Arthrobacter sp.1	++	++	+	+
Arthrobacter sp.2	+++	++	+++	+++
Acinetobacter sp.1	+++	+++	+	+
Aspergillus sp.1	+++	+++	+++	++
Aspergillus sp.2	+++	+++	+++	++
Aspergillus sp.3	++	++	++	–
Aspergillus sp4	++	+++	+	+
Penicillium sp.1	++	++	++	+
Penicillium sp.2	++	++	+++	++
Penicillium sp.3	++	++	+++	++
Penicillium sp.4	+++	++	+++	+++
Mucor sp.1	++	++	-	+
Mucor sp.2	+++	++	+++	+
Mucor sp.3	+++	+++	++	++
Fusarium sp.1	++	++	++	++
Fusarium sp.2	+++	++	+++	++
Fusarium sp.3	++	+++	++	–
Fusarium sp.4	++	++	++	–
Trichoderma sp.1	++	++	+++	++
Trichoderma sp.2	++	++	++	–
Trichoderma sp.3	++	++	+	–

Note: +++ – active development; ++ – moderate development; + – weak development; "–" – no development

Table 1 shows the ability of some active strains of microorganisms to absorb oil and oil products. The table shows that micromycetes are more active than bacteria at a temperature of 4–6 °C. The experiments performed have shown that the compounds used are suitable as the only carbon source for the growth of each isolated group of microorganisms.

5. 3. Investigation of biodegradation products at the molecular level

For the complete description of the processes of degradation of oil and petroleum products, studies were carried out on the degradation of Binagadi oil by strains of microorganisms, and then chromatography of materials was carried out. Chromatographic analysis of Binagadi oil was carried out in two stages of the initial and subsequent degradation process (after 15 and 30 days).

In the process of degradation of Binagadi oil, it was found that the chromatograms of all analyzed samples have common features and are characterized by a large number of peaks. Taking into account that Binagadi oil has a high amount of paraffinic, aromatic and naphthenic hydrocarbons, the peak values of the chromatogram, recorded first before biodegradation by microorganisms, correspond to n-alkanes, in other words, to paraffin hydrocarbons.

The following peaks corresponded to aromatic and naphthenic hydrocarbons. Such a conclusion can be reached on the basis of a chromatogram taken by a UV detector. The analysis of the mixture of the studied compounds also shows that decomposition in this chromatographic system occurs in the same order.

Then, changes in the composition and structure of petroleum products, decomposed by microorganisms, were studied under the specified conditions. At the initial stage of degradation, some changes in the peaks were observed in the zone corresponding to paraffinic hydrocarbons, easily utilizable by microorganisms. The indicated peaks were fixed before the transition of biodegradation to high-modulus forms with weakly expressed maxima. Aromatic and naphthenic hydrocarbons, difficult to digest by microorganisms, were preserved unchanged for 15 days. During subsequent cultivation (for 30 days), the degradation covered the whole mass.

Biodegradation products of oil and oil products were studied by means of IR and NMR-spectral analyses in addition to chromatological analyses. Absorption bands at 1,380, 1,460, 2,800–3,000 cm^{-1} in IR spectra are associated with deformational changes of C–H bonds in methyl and methylene groups, and bands at 1,600–1,610 cm^{-1} are associated with the presence of a benzene fragment. The presence of 960–145 cm^{-1} absorption bands indicates the presence of a naphthenic ring. The presence of absorption bands at 1,700=1,730 cm^{-1} (C=O) and 3,450–3,600 cm^{-1} (O–H) indicates the presence of a carboxyl group formed as a result of the oxidation of oil hydrocarbons by microorganisms during the biodegradation process of oil and oil hydrocarbons. The presence of absorption bands in the 740–780 cm^{-1} region in the spectra corresponds to deformational changes of CH_2 groups. In the data of NMR-spectra of biodegradation products of oil hydrocarbons, H atoms in the range of δ 6.0–8.5 m. h. characterize the aromatic structure. Changes in the interval of δ 1.4–2.5 m. h. correspond to the H atoms of hydrocarbons of the naphthenic order. Signals in the interval of δ 1.0–1.4 m. h. correspond to the CH_2 group, and those in the interval of δ 0.5–1.0 m. h. correspond to the CH_3 group of paraffin hydrocarbons. Signals of carboxyl groups chemical transitions have occurred in the interval of δ 10.02–10.65 m. h. Thus, it was determined that noticeable changes do not occur in the initial stage of degradation (during the first 15 days). After 30 days, microorganisms degrade all oil fractions and their compositions decrease and accordingly, it can be concluded that even Binagadi oil (which contains a small amount of paraffin) is degraded by microorganisms within a month.

In subsequent studies, chromatographic analyses of the decomposition of oil products (gasoline, kerosene, diesel fuel) by microorganisms were carried out. The chromatographic analyses carried out before and after the cultivation of microorganisms are shown in Fig. 3. As can be seen from Fig. 3, significant changes are observed after long-term cultivation of the studied microorganisms in oil (Fig. 3, curve 1), kerosene fraction (Fig. 3, curve 2), gasoline (Fig. 3, curve 3) and diesel fuel fraction (Fig. 3, curve 4). Microorganisms degrade all studied hydrocarbons of oil products, including paraffins, aromatics, and naphthenes, and chromatograms show faint peaks that are a clear indication of biodegradation by microorganisms.

Chromatographic and spectral analyses of oil and its degradation products have determined the oxidation sequence that shows the last aromatic fraction to be broken down. As a result, a general picture of the amount of degradation products containing carboxyl, keto-, hydroxyl groups was obtained, and this is not enough to determine the processes of transformation of initial products into final products. All these once again proves the different orientation of biodegradation processes.

As can be seen from the presented chromatograms, stronger changes occur in the composition of oil, gasoline, kerosene, diesel distillate and diesel fuel during the cultivation of the tested microorganisms. In practice, microorganisms degrade practically all hydrocarbons of the tested oil products, i.e. paraffinic, aromatic and naphthenic hydrocarbons. Only weak peaks remain on the chromatogram, which is a clear indicator of the deep biodegradation carried out by these microorganisms.

In addition to chromatographic analysis, the products of biodegradation of oil and oil products by microorganisms were analyzed by using infrared spectroscopy – IR (Fig. 4–6).

In the IR spectra of kerosene biodegradation products, absorption bands at 1,380 cm^{-1}, 1,460 cm^{-1}, 2,800–3,000 cm^{-1} were found due to deformation vibrations of C–H bonds in methyl and methylene groups, and bands at 1,400–1,720 cm^{-1} are characteristic of hydroxyl and carboxyl groups. In the spectra, the absorption bands found in the region of 740–780 cm^{-1} correspond to the frequency of bending vibrations of CH_2 groups. Also, absorption bands characteristic of aromatic rings and double bonds (1,600–1,660 cm^{-1}), absorption bands characteristic of the aldehyde group were found at 1,685 cm^{-1}.

In the IR spectrum of gasoline biodegradation products, the bands in the region of 2,960–2,950 cm^{-1} are characteristic of deformation vibrations of C–H bonds, and the bands in the region of 1,400–1,450 cm^{-1} are characteristic of the naphthenic fraction. At the same time, absorption bands characteristic of the aldehyde group were found at 1,685 cm^{-1}. Absorption bands at 1,600–1,660 cm^{-1} are characteristic of the aromatic ring and the double bond.

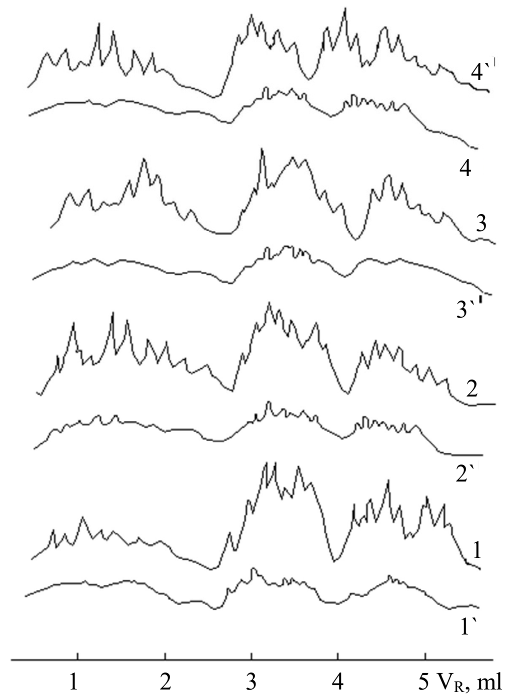

Fig. 3. Adsorption chromatogram of oil fractions before biodegradation (1–3) and after biodegradation (after 30 days) by active strains of microorganisms: 1 – oil (1,1'); 2 – kerosene (2,2'); 3 – gasoline (3,3'); 4 – diesel fuel (4,4')

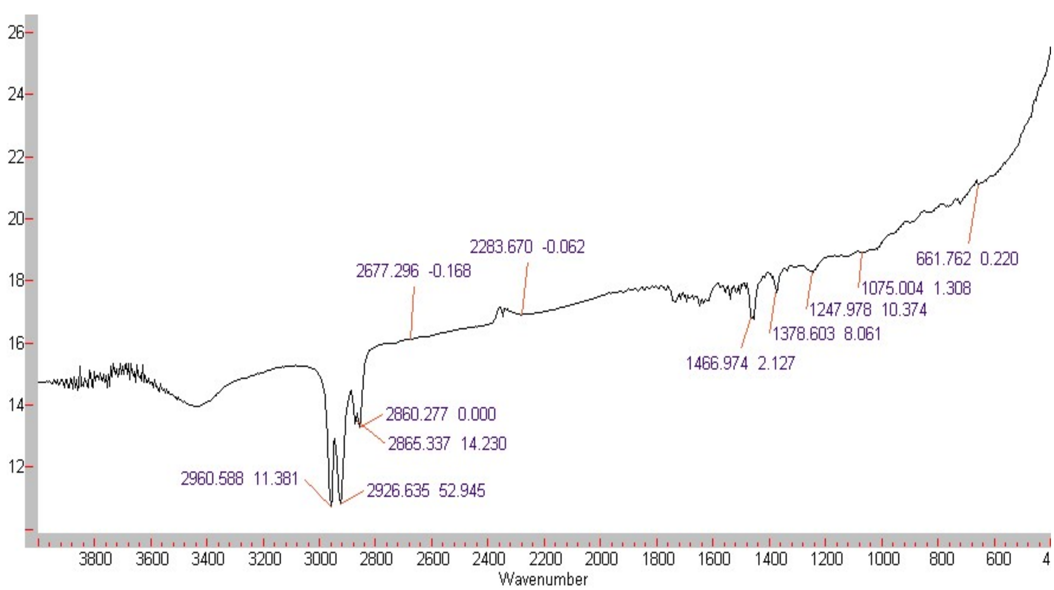

Fig. 4. Infrared spectrum of kerosene after degradation by associations of microorganisms

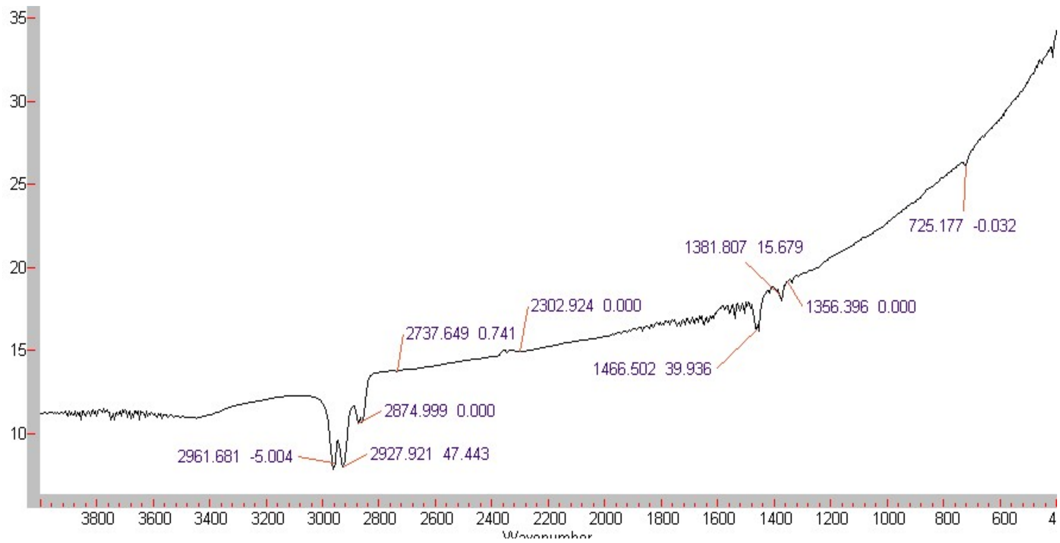

Fig. 5. Infrared spectrum of gasoline after degradation by associations of microorganisms

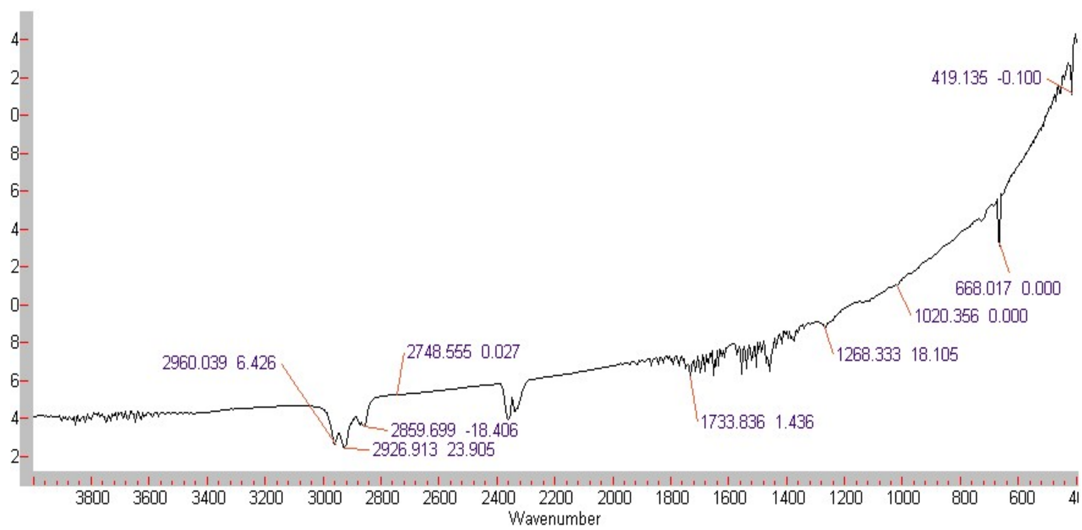

Fig. 6. Infrared spectrum of oil after degradation by associations of microorganisms

In the IR spectrum of oil biodegradation products, the absorption bands at 1,380, 1,460, 2,859–2,960 cm^{-1} are due to deformation vibrations of C–H bonds in methyl and methylene groups. The presence of absorption bands with frequencies of 960–1,450 cm^{-1} indicates the presence of a naphthenic ring. The presence of absorption bands in the region of 1,690–1,730 cm^{-1} (C=O) and 3,450–3,600 cm^{-1} indicates the presence of carboxyl groups in the composition of oil degradation products as a result of the oxidation of oil hydrocarbons. At the same time, absorption bands characteristic of the aldehyde group were found at 1,685 cm^{-1}.

6. Discussion of the results of selection and use of microorganisms-destructors of oil and petroleum products at low positive temperatures

As a result of the research, it was found that strains of *Bacillus, Micrococcus, Pseudomonas, Arthrobacter, Acinetobacter, Aeromonas, Vibrio, Sarcina, Alcaligenes* and micromycetes *Aspergillus, Penicillium, Mucor, Fusarium, Trichoderma*, isolated from the shores of the Caspian Sea, actively assimilate oil and oil products. As can be seen from Table 1, the best substrate for the investigated micromycetes was oil, kerosine, gasoline. Worst of all, microorganisms absorbed diesel fuel.

And it was also found that micromycetes are more active than bacteria at a temperature of 4–6 °C. As shown in Fig. 1, 2, 96 % of micromycetes and 70 % of bacteria isolated from the waters of the Caspian Sea used oil as the only source of energy and carbon. At a temperature of 4–6 °C, the weakest development was observed in fuel oil. The experiments performed have shown that the compounds used are suitable as the only source of carbon for the growth of each isolated group of microorganisms.

The process of transformation of the studied compounds by the indicated microorganisms in all cases began in 12–14 days. At the same time, the formation of intermediate products continued for 10–12 days. During this time, on the chromatograms of the starting compounds, depending on the exposure time, a decrease in the intensity of their signals recorded by the UV detector, in parallel, an increase in the intensity of the signals of the resulting biotransformation products were observed. It should be noted that the figures show only the final stage of the transformation of the studied compounds, the composition of which remains stable for a certain period of time (up to

20–25 days). This period is important for processing the resulting individual compounds. It has been found that up to 30 days they are sharply utilized until the corresponding signals on the chromatograms completely disappear.

As can be seen from Fig. 3, hydrocarbons of the paraffin series are decomposed first. The conversion of aromatic hydrocarbons and naphthenic hydrocarbons begins after 15 days. After 30 days, microorganisms decompose all fractions of oil and their composition decreases, so it can be concluded that even Binagadi oil, which contains a small amount of paraffin, is decomposed by microorganisms within a month.

Stronger changes occur in the composition of oil, gasoline, kerosene and diesel fuel during the cultivation of tested microorganisms. Practical microorganisms degrade almost all hydrocarbons, tested petroleum products, etc. paraffin, aromatic and naphthenic hydrocarbons. Only weakly expressed peaks remain on the chromatogram, which is a clear indicator of deep biodegradation carried out by these microorganisms (Fig. 3).

On the basis of spectral analysis, the sequence of degradation of oil and oil products was determined, it was found that aromatic hydrocarbons are the last to decompose (Fig. 4–6).

The ability of micromycetes to biodegrade oil and oil products indicates that these microorganisms are widespread in the Caspian Sea and take part in the self-purification of the aquatic environment from oil pollution.

Research in this direction is ongoing, these abilities of micromycetes should be studied in broader aspects in connection with the possibility of their use for solving problems related to the biological treatment of polluted areas of the Caspian Sea.

Obviously, the methods of biological water purification from oil products have both advantages and disadvantages, which opens up opportunities for their improvement. Thus, the proposed approach allows us to draw conclusions about the advantages of the biological cleaning method, which is its environmental safety. The main disadvantage of most biological wastewater treatment methods is the difficulty in maintaining the bacterial population and maintaining their activity.

The results obtained do not contradict the data of the works [12, 14]. It has been shown that for the biological purification of waters from oil and oil products, the use of strains of oil-destroying microorganisms contributes to a significant acceleration of the destruction of oil products. However, there are some restrictions on the growth of microorganisms, limited by the lack of oxygen, nitrogen and phosphorus, which requires the use of this method in the conditions of the autumn-spring period. These restrictions are due to the fact that in the colder season, cleaning up from oil pollution becomes more difficult and at the same time significantly increases its cost. The development of research can be carried out along the lines of overcoming the noted limitations and shortcomings. Using our research methodology, you can get a significant effect in the field of cleansing the Caspian Sea from pollution by oil and oil products.

7. Conclusions

1. Oil-oxidizing bacteria from the genera *Micrococcus, Bacillus, Pseudomonas, Arthrobacter, Mycobacterium, Acinetobacter, Aeromonas, Vibrio, Sarcina, Alcaligenes* and mycomycetes from the genera *Penicillium, Aspergillus, Fusarium, Mucor, Trichoderma* were isolated from the selected waters of the coastal territories of the Caspian Sea. Of the isolated strains of micromycetes, 96 % grew on oil, 82 % on kerosene, 60 % on diesel fuel, and 54 % on gasoline.

Bacterial strains absorbed oil and petroleum products worse than micromycetes. Of the isolated strains, 70 % developed in oil, 43 % in kerosene, 58 % in diesel fuel, and 34 % in gasoline.

2. Conducted experiments showed that isolated microorganisms from the Caspian Sea are capable of destroying oil and petroleum products at a temperature of 4–6 °C. Because associations from the genera of bacteria *Bacillus, Micrococcus, Pseudomonas, Acinetobacter and Arthrobact*er and from the genera of fungi *Aspergillus, Trichoderma, Penicillium, Mucor* are capable of absorbing oil, gasoline, kerosene, diesel fuel at a temperature of 4–6 °C. The studied types of micromycetes and bacterial-fungal associations can be used to create an active biopreparation based on indigenous microorganisms with further use for bioremediation of water, as well as treatment of tanks and other containers used for long-term storage of oil products.

3. Almost all microorganisms isolated from the waters of the Caspian Sea decompose oil and oil products, i.e. paraffinic, aromatic and naphthenic hydrocarbons. A general picture of the amount of degradation products containing carboxyl, keto, hydroxyl groups was obtained, which is not enough to determine the processes of transformation of initial products into final ones. All this once again proves the different directions of biodegradation processes.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

Financing

The study was performed without financial support.

Data availability

The manuscript has no associated data.

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